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The Alveolar Macrophage

by Drummond H. Bowden*

The pulmonary macrophagic system is critical to the defense of the lung, keeping the alveoli clean and sterile and responding on demand with an adaptive outpouring of new cells into the air sacs. Under basal conditions alveolar macrophages, in common with other mononuclear phagocytes, are derived from the bone marrow. A population of macrophage precursors within the pulmonary interstitium provides a reserve pool capable of proliferation and delivery of phagocytes in response to unusually heavy loads of inhaled particles. This reserve system also produces macrophages when monocytic precursors in the bone marrow are depleted by diseases such as leukemia.

The alveolar macrophage is destined to ingest particulate matter and to be eliminated along the mucociliary pathway; clearance by lymphatics is of minor importance and macrophages probably do not recross the alveolar epithelium to reach the pulmonary interstitial compartment.

Although the protective role of the macrophage is dominant, this cell may participate, directly or indirectly, in the genesis of two major groups of chronic pulmonary disease, interstitial fibrosis and emphysema. Such inappropriate responses involve interactions with fibroblastic cells and tissue injury initiated by proteases secreted by the macrophage.

Introduction

The sputum of a healthy person contains a fair number of large mononuclear cells containing cytoplasmic inclusions. The sputum of an individual working in an excessively dusty environment yields many more of these cells, and they are packed with dust particles. These simple clinical observations suggest a mechanism whereby the lungs are kept clean and provide a clue to the relative infrequency of pulmonary disease in relationship to the constant bombardment of the respiratory system with toxic, infectious and allergenic particles. From the outset one cannot fail to be impressed by the capacity of the pulmonary defenses to cope with most environmental stresses and to adapt to changing atmospheric conditions.

In the overall protection of the lung the alveolar macrophage plays a pivotal role, beyond the membranes of the nose and the mucociliary escalator, the ultimate scavenger that keeps the alveoli clean and sterile and responds on demand with a rapid and massive outpouring of new cells into the air sacs. As part of the mononuclear phagocytic system of the body, the alveolar macrophage is an essential component in the immunologic chain. But, whereas the tissue macrophage is concerned mainly with processing and transference of antigens to lymphoid receptors, the free phagocyte of the lung is involved primarily with phagocytosis and clearance, keeping the air sacs free from inhaled particulate antigens which may cross the epithelial barrier, reach the pulmonary interstitium and proceed thence to the lymphoid receptors of the lung. The major, though not exclusive, immunologic role of the alveolar macrophage appears to be protection of the pulmonary lymphoid cells from allergenic overload.

Finally, there is increasing evidence supporting a function for the pulmonary macrophage in initiating or at least mediating such important diseases of the lung as emphysema and interstitial fibrosis. This essay presents a profile of the alveolar macrophage, relating structure with function, cellular kinetics with adaptive responses and toxicologic responses to the production of pulmonary disease.

Origin and Kinetics

The familial unity of the cells of the mononuclear phagocytic system and their ultimate derivation from a precursor in the bone marrow is generally agreed. Much of the evidence favoring this hypothesis is based upon studies of monocytic egress during inflammation (*1*) and of mononuclear kinetics in radiation chimeras (*2–4*). Whether a similar mechanism operates under resting or basal conditions is not known. Volkman (*5*) favors a local origin for hepatic and peritoneal macrophages, a view that is sharply challenged by van Furth (*6,7*) who stoutly defends the supremacy of the monocyte. The two views are neither irreconcilable nor mutually exclusive. Certainly, with respect to the macrophages of the lung, the operation of a dual monocytic and interstitial system is established. This system supports the basal requirements of the lung and responds quickly and effectively to increased functional demands (*8–12*).

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Figure 1 depicts the duality of the macrophagic delivery system and also poses an unresolved question. The origin of circulating monocytes from a population of dividing promonocytes in the bone marrow is known. Monocytes leave the circulation at random but the final pathway from blood to alveoli is uncertain. There are at least four possibilities: (1) direct migration of monocytes across the endothelium, interstitium and epithelium to reach the alveoli; (2) migration of monocytes or marrow-derived colony-forming units (CFU) into the interstitial tissues where they divide and possibly mature before entering the alveoli; (3) division of resident macrophages with subsequent migration into the air sacs; (4) combinations of (1)–(3).

Under certain abnormal situations both monocytes and interstitial cells may contribute to the output of free alveolar macrophages; for instance, in leukemic patients, the number of alveolar macrophages is maintained despite severe depletion of monocytes; similarly in the experimental situation, if monocytes are depleted *in vivo* by irradiation (*13*) or *in vitro* in an organ culture system (*10*), alveolar macrophage production is maintained by proliferation of interstitial cells. On the other hand, a heavy load of inhaled particulates induces a rapid efflux of monocytes into the alveoli, followed by a sustained increase in mitotic activity within the interstitium of the lung (*9–11*).

In his early studies, van Furth (*7*) gave a loading dose of ^{3}H-thymidine to normal animals, thereby ensuring heavy nuclear labeling of all monocytes leaving the bone marrow. He measured the rate of disappearance of heavily labeled monocytes from the blood and calculated the half-time of monocytes in the circulation as 22 hr. Recently, we have used this technique to measure the transit times of monocytes in the blood, the pulmonary interstitium and the alveoli of the lung (*12*). The results are summarized in Figure 2.

On a semilogarithmic scale the rate of disappearance of labeled mononuclear cells from the blood is linear, with a half-time in the circulation of about 24 hr; in the interstitium the half-time is 2 days and in the alveoli 4 days. Of particular interest are the differences between the lines for monocytes and those for the interstitial

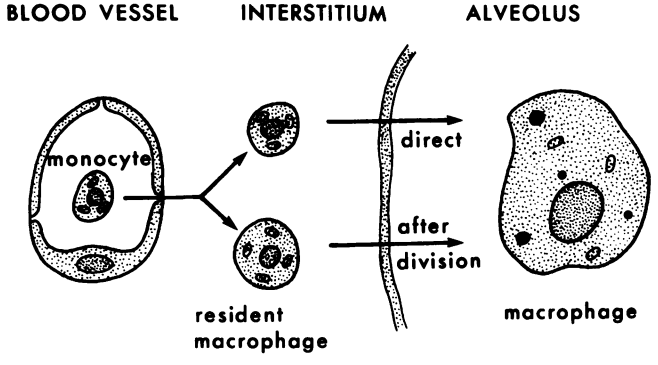

FIGURE 1. Dual origin of alveolar macrophages. From Bowden and Adamson (*12*).

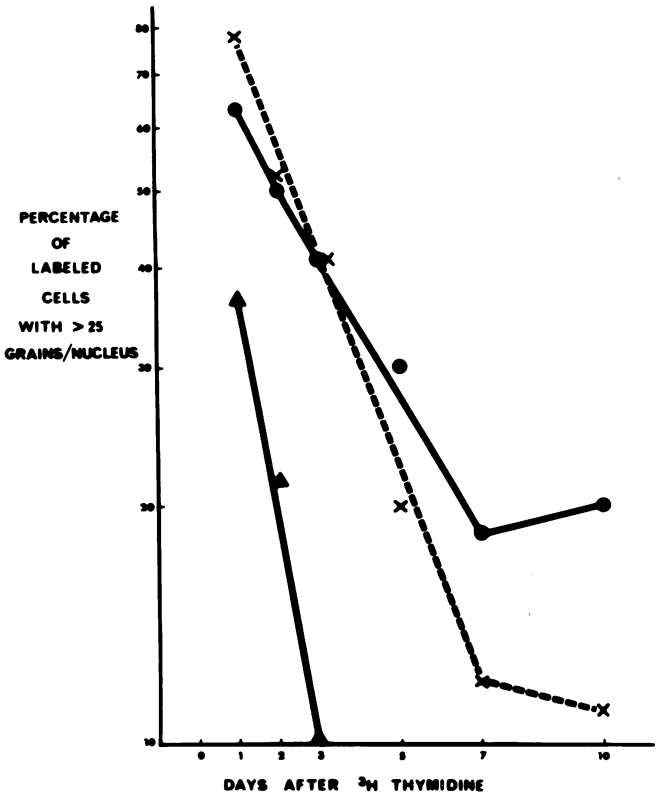

FIGURE 2. Transit times of monocytes in blood, pulmonary interstitium and alveoli as measured by the rate of disappearance of thymidine labeled cells (> 25 grains) after ^{3}H-thymidine injections: (▲) monocytes; (X) interstitial cells; (●) alveolar macrophages. From Bowden and Adamson (*12*).

cells and free macrophages. The rates of disappearance of labeled interstitial cells and macrophages are exponential to day 7 and roughly parallel to the monocyte graph. Subsequently, however, the monocyte graph is exponential to zero, whereas the lines for interstitial cells and macrophages diverge after day 7 due to the persistence of a small number of heavily labeled cells. The biphasic nature of the lines suggests that although, under basal conditions, the majority of alveolar macrophages arise by the fairly rapid migration of monocytes across the interstitium, there is a slower component that cannot be explained in this way. Further studies of mitotic rates and sequential grain counts in the interstitial and alveolar compartments suggest that a small proportion of alveolar macrophages is derived by division of resident interstitial cells (*12*). Upon demand, both monocytic and interstitial components of the delivery system produce an integrated response to meet the changing needs of pulmonary defense (*9,11*).

Multiplication of free macrophages within the alveoli can occur although, under normal circumstances, it is unusual. These functional alveolar cells are in the G^0 phase of the mitotic cycle, they are rarely labeled after a pulse of ^{3}H-thymidine and in conventional cultures no increase in cell number occurs. Cell division after

culture in soft agar has been reported (*14*) and several investigators have been able to induce mitotic activity in cultured macrophages by adding various "conditioned" media (*15*) or cell-free inflammatory exudates (*16*); *in vivo*, division of alveolar macrophages has been demonstrated after exposure to nitrogen dioxide (*17*). These observations indicate the capacity of at least some free alveolar cells to respond to a variety of mitogenic stimuli. Under basal conditions, the percentage of dividing alveolar macrophages in a 24-hr period does not exceed 0.12% (*9*). Whether these dividing cells are regular macrophages or colony forming units (CFU) which may, under suitable conditions, be stimulated to increase their rate of division, is not known.

In summary, it can be stated with confidence that delivery of macrophages to the air sacs involves two pathways; the majority arrive by direct passage of monocytes from capillaries to alveoli, a smaller number arise by division of resident interstitial cells which then migrate to the alveoli.

Form and Function

Certain features, related to phagocytosis and lysosomal digestion are common to macrophages in all parts of the body. In this respect, although alveolar macrophages conform to the general pattern, the restricted view of these cells gained from microscopic sections of the lung provides only a fragmentary insight into their morphologic variety. A more complete picture is obtained by examining cells recovered by bronchoalveolar lavage which yields some 3 to 15 million macrophages per gram of lung (*18*).

The morphologic heterogeneity of the lavaged cells is dependent upon the ages of the cells, the phases of cellular activity and the quantity and quality of materials that have been phagocytosed. Immature cells contain abundant smooth endoplasmic reticulum whereas actively working cells exhibit much rough endoplasmic reticulum and many lysosomes. Phagosomes may contain particles of dust, bacteria, cellular debris and lamellar configurations derived from Type II cells as they spill surfactant onto the alveolar surface.

The structural heterogeneity of the alveolar macrophage reflects its metabolic and synthetic activities. In common with other macrophages the alveolar phagocyte rapidly synthesizes lysosomal enzymes. Sorokin (*19*) has described a branched system of smooth-surfaced tubules within the cytoplasm of the alveolar macrophage. This membranous array appears to be identical with the specialized lysosome-producing system, GERL (Golgi apparatus, endoplasmic reticulum, lysosomes) described by Novikoff et al, (*20*) in 1971. The prominence of this membranous array in the alveolar macrophage may represent a mechanism whereby the cell is able to respond rapidly to demands for synthesis and packaging of acid hydrolases under load conditions. (Fig. 3)

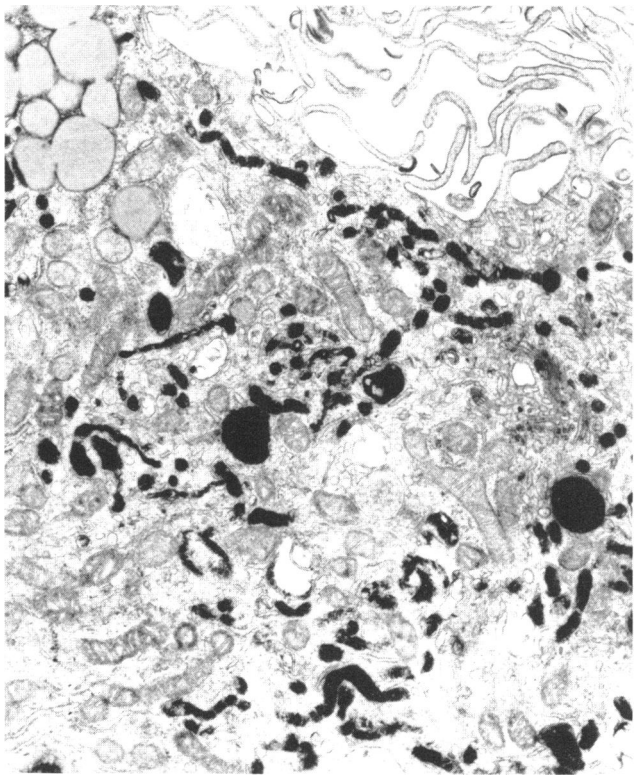

FIGURE 3. Acid phosphatase preparation demonstrating enzyme activity in the tubular array of lysosomes in an alveolar macrophage.

The increased levels of lysosomal enzyme activity in alveolar macrophages as compared with other mononuclear phagocytes is related to functional demands placed upon the alveolar cell because of its direct exposure to the environment. The importance of microbial phagocytosis in the initiation of enzyme synthesis in the alveolar cell has been emphasized by Heise and his associates (*21,22*). Macrophages obtained from germ-free animals have a much lower level of enzymatic activity than those recovered from animals raised in nonsterile conditions; similarly, lysosomal enzyme activity is greater in the fall and winter than in spring and summer. In addition to enzyme synthesis related to phagocytosis and lysosomal digestion, the alveolar macrophage, like its counterparts in other tissues, produces a variety of proteolytic enzymes, including elastases and collagenases, which are secreted externally (*23*, *24*).

The relationship between macrophagic form and function is displayed most convincingly in the developing lung. In the rat, which has a gestation of 22 days, alveolar macrophages are first observed only at day 20; these cells have few pseudopodia, lysosomes are scanty and, although there is abundant material such as lamellar bodies available in the terminal sacs, the young macrophages are reluctant phagocytes (*25*). Subsequently, as phagocytic activity increases, large quantities of phospholipids and other materials are seen in the phagosomes.

The immediate postnatal period is characterized by a

dramatic increase in the number of alveolar macrophages (*26*), a response which appears to be related to the relatively high mitotic rate of interstitial cells as new alveoli develop (*27*). The ability to phagocytose and to kill bacteria also increases rapidly with age; by 4 weeks, rabbits attain adult levels of bactericidal activity (*28*).

As macrophagic function develops, morphologic changes indicative of maturation occur; a well-developed Golgi system and rough endoplasmic reticulum is observed together with an increase in the number of mitochondria and lysosomal bodies (*26*). Cytochemically, the acid phosphatase activity of macrophages in 10-day-old rats is much increased over that observed in prenatal animals (*29*). Such findings suggest that the incomplete development of the macrophagic system at birth may be a factor in the susceptibility of the newborn to infection. In this respect the fetal macrophage in its sterile environment resembles the postnatal macrophage of the germ-free animal. Functional maturation of the macrophagic system appears to be an innate adaptive response to the sudden change from a sterile intrauterine environment to an external milieu of the ambient air.

Adaptive Responses

The heterogeneity of the macrophagic population obtained by bronchoalveolar lavage reflects the capacity of these cells to adapt to a changing environment (Fig. 4). The newly arrived cell in the alveolus is small, much like a blood monocyte; in a sterile milieu such as the fetal lung or in the germ-free animal with little or no stimulus to phagocytosis, the cell remains unaltered. Challenged by microbial or other particulates the macrophage adapts rapidly, synthesizing new membranes and lysosomal enzymes. There are corresponding morphologic changes and the older functioning cell shows many phagosomes, a prominent Golgi and much rough endoplasmic reticulum (Figs. 3 and 4).

This process of individual cellular adaptation combined with relatively small fluctuations in the number of alveolar macrophages, keeps the air sacs sterile and free from potentially harmful particles and cellular debris. But there is need also for the system to respond rapidly and effectively to massive overload or to a steady and prolonged increase in the burden of inhaled particulates. There is such a mechanism and it is a most efficient mechanism because, despite constant bombardment by a variety of particles, injury to the distal respiratory units is an unusual event.

Within certain limits, there is a direct relationship between the output of alveolar macrophages and the effective particulate load reaching the air sacs (*30*). There is a limit beyond which the system may be saturated but, except for a few unusual circumstances such as sudden exposure to millions of spores and other organic particles in a dusty barn, the mechanism works well. Such an arrangement, whereby cellular output is programmed to meet functional demand, requires a

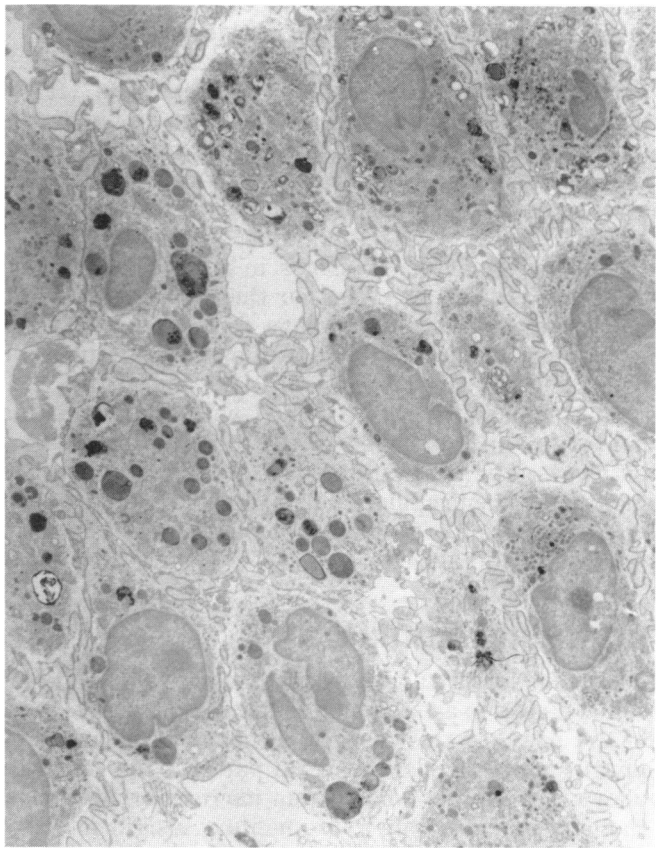

FIGURE 4. Alveolar macrophages lavaged from the lungs of a healthy mouse. Cellular heterogeneity is related to the variable number, size and content of phagolysosomes.

finely tuned control mechanism. Although very little is known about the control of the system, the pathways of cellular delivery are now fairly well established.

Under basal conditions alveolar macrophages arise from two sources; most are derived frm blood monocytes, a smaller proportion from a slowly dividing population of interstitial cells (*12*) (Fig. 1). Under load conditions, increased macrophagic output is achieved by an acceleration of the normal biphasic system (*9*). The magnitude of the cellular response to a large load is impressive. Pulmonary instillation of 4 mg of carbon (300 Å particles) into the lungs of a mouse induces a massive response within a few hours and by 24 hr the number of macrophages obtained by lavage has increased 10-fold (Fig. 5). This initial outpouring of macrophages precedes any increase of cellular division within the pulmonary interstitium (*11*). At this early time, monocytes crowd the small vessels of the lung, cross the endothelial barrier of the venules, the interstitium and the alveolar lining to reach the air sacs (Fig. 6). Within the alveoli the new arrivals closely resemble monocytes; they are small with a paucity of membranes and despite the abundance of carbon in the air sacs, they show little tendency to phagocytosis (Fig. 7). Subsequently, monocytic crowding of the microcirculation disappears. Main-

tenance of a high level of macrophagic output appears to be dependent upon increased mitotic activity of mononuclear cells within the interstitial compartment (Fig. 8).

In summary, the adaptive increase in macrophagic output in response to a large particulate load is biphasic. Initially, blood monocytes pour out of the venules and enter the alveoli; later, increased mitotic activity of pre-existing or newly arrived mononuclear cells in the interstitium provides a continuing supply of cells (*9,11*). Even under heavy stress, division of free alveolar macrophages makes no significant contribution to this adaptive response (*12*).

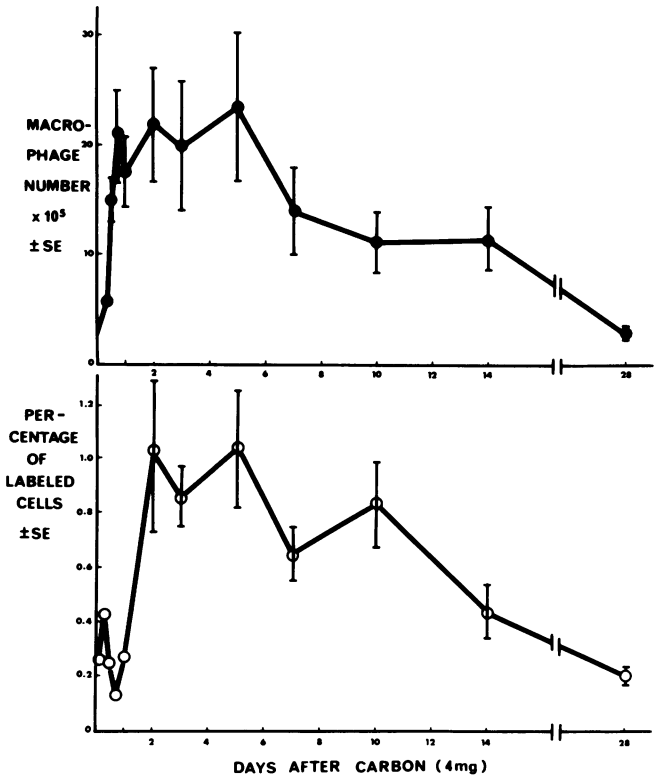

FIGURE 5. Plots of (top) the number of alveolar macrophages recovered from mouse lungs at intervals after carbon instillation and (bottom) percentages of ^{3}H-thymidine-labeled cells in autoradiographs. DNA synthesis in the lung is not elevated at 1 day but, from 2 days on, it correlates well with the increased number of macrophages.

Dose Response

The macrophagic delivery system appears to be finely tuned to the effective burden of small particles reaching the alveoli (*30*). Output may be influenced by the magnitude of the load, its chemical composition and the size of the individual particles. Such variability of response could be explained by the relative predominance of either the monocytic or interstitial pathways of recruitment. In the experimental models studied to date, the early monocytic response is transient whatever the dose. It is the duration of the interstitial cell response that determines the continuing outpouring of phagocytes; this phase is dose dependent (*31*). A clear dissection of the two components of macrophagic production is show in Figure 9. Instillation of 0.1 mg carbon induces no increment of macrophages; after 1.0 mg the response is brisk but short-lived, whereas the maximal yield induced by 8.0 mg is maintained for several days and at 14 days is still some five times greater than normal.

Autoradiographic studies indicate a transient increase in pulmonary DNA synthesis after 1 mg; with the bigger loads of carbon, the prolonged increase in macrophagic numbers is accompanied by a 3-fold increase in mitotic activity in the pulmonary interstitium which persists for at least 10 days (*31*). The monocytic and interstitial limbs of the delivery system are coordinated to produce an integrated response to alveolar loading; the former is rapid but transient, the latter is slower but persisting.

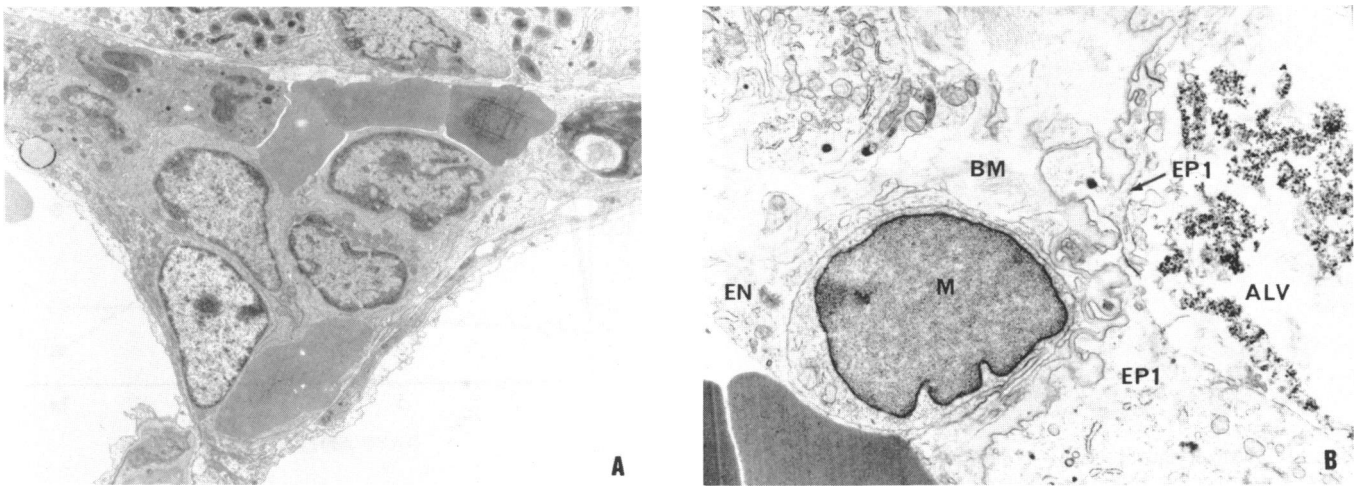

FIGURE 6. Micrographs taken (*A*) 6 hr after carbon; monocytes crowd a small pulmonary vessel; (*B*) 12 hr after carbon; a monocyte (M) is observed between endothelial cells (EN) of a pulmonary vessel. Free carbon is seen in a neighboring alveolus (ALV); B.M. = basement membrane; EPI = Type I cell. From Adamson and Bowden (*8*).

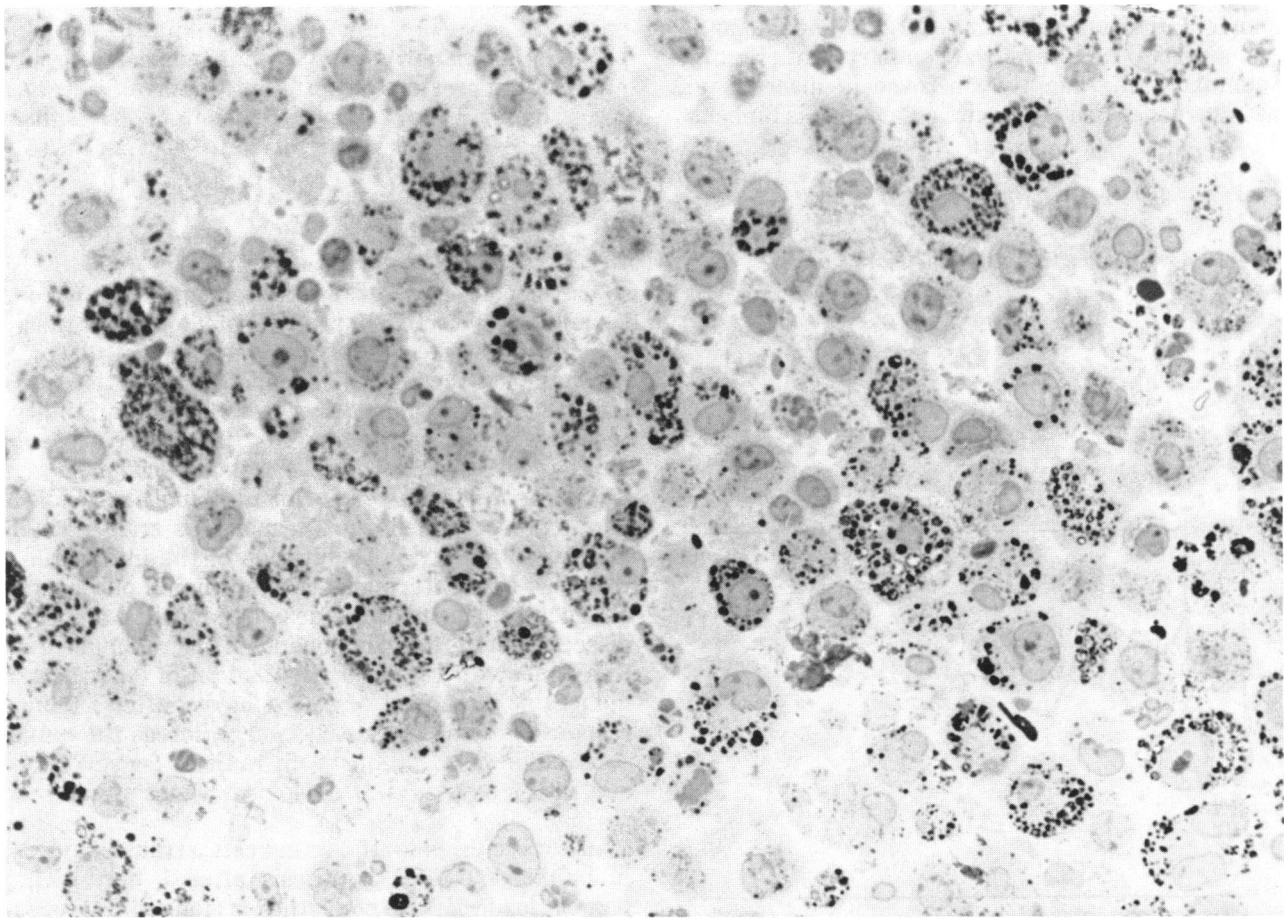

FIGURE 7. Cell pellet (0.5-μm section) of macrophages lavaged from mouse lung 3 days after intratracheal carbon. Many cells are large and loaded with carbon; others, probably new arrivals, are small and contain no carbon.

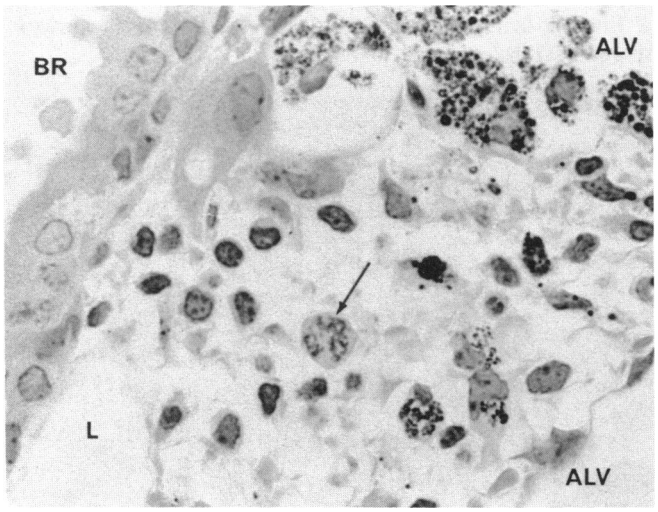

FIGURE 8. Methacrylate section, 5 days after carbon. Alveolar macrophages are large and packed with carbon. The interstitial macrophages in the peribronchial space are smaller and contain few carbon particles. A mononuclear cell in mitosis is also shown (arrow). BR = bronchial epithelium; L = lymphatic; ALV = alveolus.

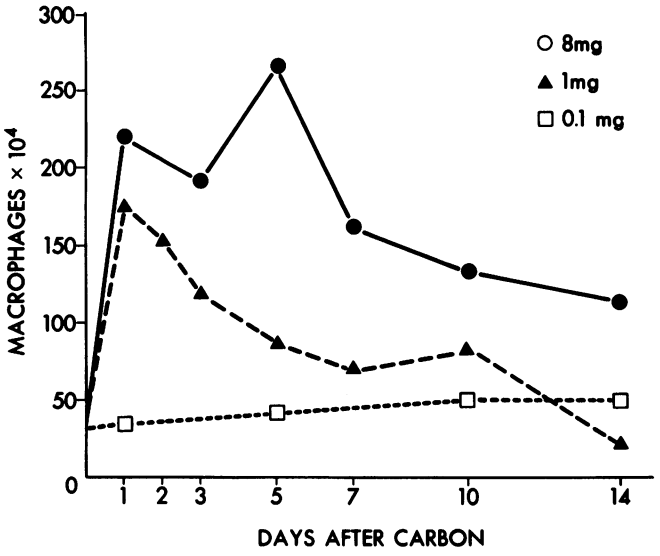

FIGURE 9. Plots of the number of alveolar macrophages recovered from mouse lungs at intervals after the instillation of different weights of carbon.

The effective stimulus that triggers these responses appears to be related more closely to the number of particles instilled than to the total load. Small particles of carbon or coal dust induce a much greater macrophagic response than is found with identical loads of much larger particles (*30*). A similar response is observed with microbial organisms of different sizes and following instillation of the same weight of carbon or latex particles varying in size from 0.03 μm to 1.0 μm (*31*) (Fig. 10). The tiny particles of carbon provoke a macrophage yield at least three times greater than a similar load of latex spheres and there appears to be a direct relationship between the number of particles and the duration of enhanced macrophagic production by the cells of the pulmonary interstitium. Although this interstitial response is dose dependent, lower and upper thresholds have been identified. In the murine lung, delivery of 10^{10} particles is needed to stimulate cellular division in the interstitium; with particulate numbers above 10^{13} the adaptive capacity of the system appears to be saturated (*31*).

The importance of the dual pathways of macrophagic supply to the alveoli is further emphasized by the ability of monocytopenic individuals to maintain at least basal numbers of macrophages within the alveoli. When circulating monocytes are absent as in patients with leukemia, macrophagic output is maintained, presumably by the cells of the interstitial compartment (*32*). It is not known if such individuals are capable of mounting an appropriate macrophagic response to an increased inhalant load. It is possible, however, to mimic this situation in animals depleted of monocytes by irradiation of the bone marrow. Following irradiation the output of macrophages is maintained, and when these animals are given a heavy load of carbon, macrophage output doubles within 1 day and is tripled by the end of 2 weeks (*33*). Figure 11 illustrates the comparative responses of irradiated and nonirradiated animals given identical particulate loads. The normal animals exhibit a 10-fold increase of macrophages within 24 hr; with the irradiated animals the number of free cells obtained by lavage is much lower. In the absence of circulating monocytes the adaptive response is limited and explained entirely by increase mitotic activity in the interstitial compartment of the lung.

The validity of the biphasic hypothesis of macrophagic production and delivery is firmly supported by these various models of macrophage kinetics. The blood monocyte provides the main source of alveolar macrophages; the interstitial compartment which, under basal conditions, makes only a small contribution to the macrophagic pool, provides an essential back-up mechanism capable of responding to particulate loading in normal and in monocytopenic individuals.

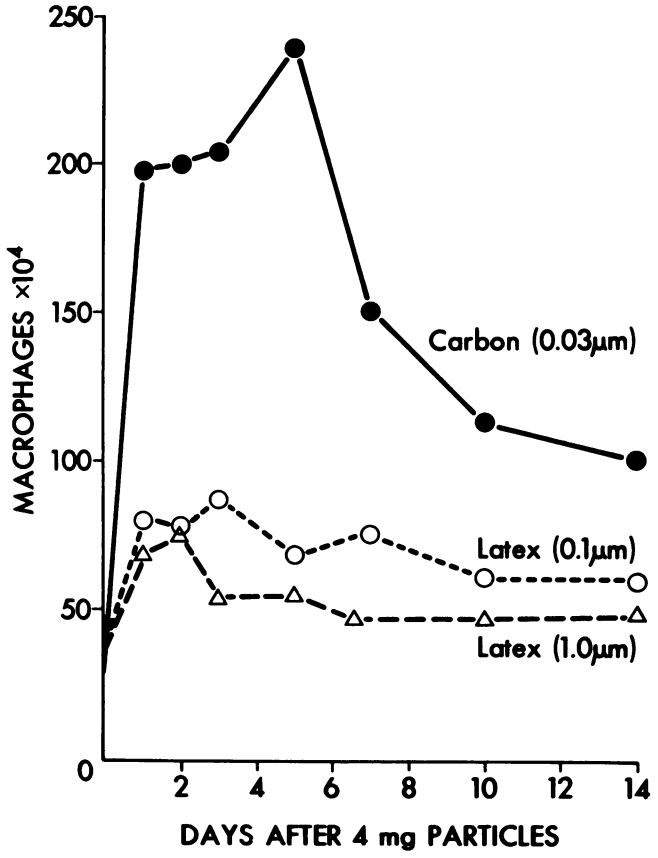

FIGURE 10. Plots of the number of alveolar macrophages recovered from mouse lungs at intervals after the instillation of equal weights of particles of different sizes.

Control of Macrophage Recruitment

The outpouring of macrophages that follows the arrival of a particulate load in the air sacs occurs very rapidly and reaches its peak at least 24 hr before the

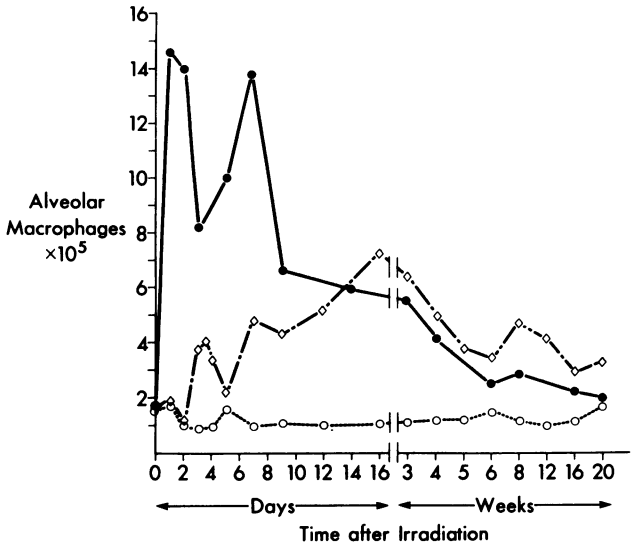

FIGURE 11. Plots of the number of alveolar macrophages recovered from mouse lungs under various conditions: (•) 4 mg carbon, no irradiation; (○), irradiation, no carbon; (◇) irradiation followed by 4 mg carbon at day 2.

interstitial cells start to proliferate (*11*). The speed of this response and the accompanying, although transient, efflux of large numbers of neutrophilic leukocytes suggest chemotactic recruitment into the alveoli (*8,11*). The nature of the chemotactic stimulus is not known and although macrophages in culture release factors which are powerful neutrophilic attractants, their ability to stimulate macrophagic cells is less marked (*34–36*). Also unexplained is the brief duration of the neutrophilic response although particles and particle-laden macrophages persist. The continuing output of macrophages which involves division of interstitial cells, suggests mitogenic stimulation at this site. The mitogen is not known although the temporal proximity of the arrival of free particles in the interstitium and the onset of cellular division at least suggests a causal relationship (*8,11*).

The cellular response to inhalation of cytotoxic particles may provide a clue to the mechanisms that control macrophage production. Silica induces a much larger macrophagic outflow than is produced by similar numbers of less toxic particles such as ferric oxide or titanium dioxide. Privalova et al. (*37*) ascribe this augmented response to the action of cellular products released into the alveoli by the lysis of silica containing macrophages. These products of macrophagic breakdown appear to signal recruitment of macrophages and even greater numbers of neutrophilic leukocytes by systemic and local stimulation of macrophagic and granulocytic production.

Pulmonary Clearance

As the ultimate scavenger of the lung the macrophage is destined to be eliminated. The life-span of this cell within the alveolus is related to functional demand; under basal conditions the half-time of the macrophage in the alveolar compartment is about 4 days (*12*); under sterile conditions it may be longer, although this has not been determined. In response to a heavy load of inhaled particulates, the transit time through the alveolus may be halved with a continuing supply of young macrophages pouring into the air spaces from the monocytic and interstitial compartments (*9*). Such measurements of the speed of transit through the air sacs tell us nothing about the potential life-span of the macrophage. In tissue culture these cells are viable for more than 100 days, and when pulmonary clearance is impaired the alveolar macrophage appears to survive for periods as long as 3 months.

The airways provide the major, if not the exclusive, route of macrophagic clearance. The initial step in this process involves movement of cells from the air sacs to the openings of the respiratory and terminal bronchioles. How this movement occurs is not known; respiratory movements and elastic recoil undoubtedly are important and passage may be aided by the flow of an alveolar surface film (*38*). Once on the bronchiolar epithelium the macrophages are transported by ciliary movement to the pharynx where they are swallowed (Fig. 12).

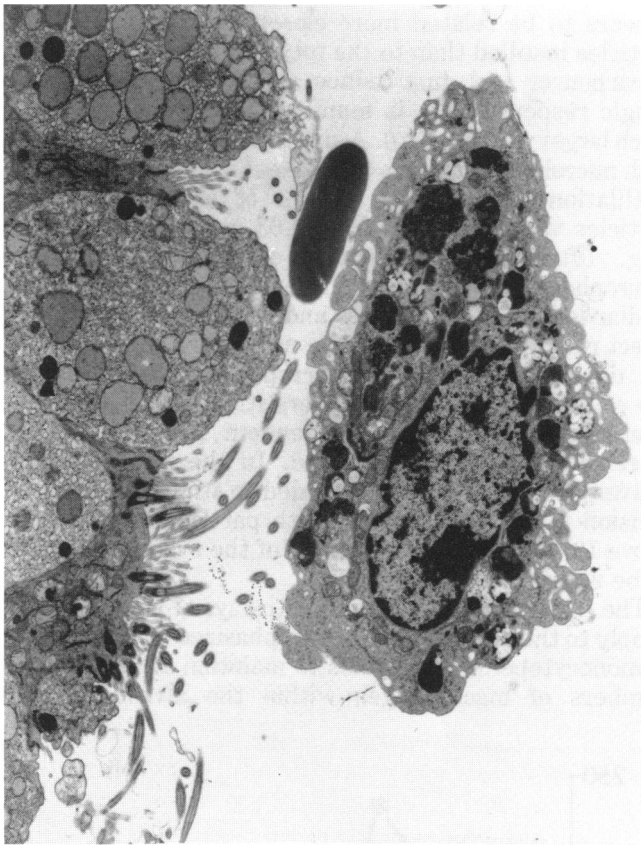

FIGURE 12. Surface of bronchiolar epithelium showing a macrophage laden with phagocytosed material.

Alveolar macrophages may be cleared by other routes although the evidence favoring this is far from convincing. Some maintain that macrophages with their ingested loads recross the epithelium to enter the interstitium whence they proceed to lymphatics and their draining lymph nodes. The presence of particle laden macrophages within the interstitial tissues is cited in support of this hypothesis. In the light of recent observations it seems more likely that particles cross the thin squamous alveolar epithelium in the free state; within the interstitium they are phagocytosed by interstitial macrophages (*8*). Under most conditions, however, the barrier between the air sacs and the interstitium is not breached. The functional reserve of the macrophagic system with its pool of interstitial cells and a continuing supply of feeder monocytes in the pulmonary circulation is more than adequate to handle most inhalant loads. Pathologic changes supervene when the adaptive capacity is overwhelmed.

The upper limit to the adaptive increase of macrophages is illustrated by the carbon loading experiments (Figs. 9–11). Beyond this threshold increasing the number of free particles in the alveoli enhances the likelihood of passage across the alveolar epithelium to reach the interstitium (*31*). Carbon particles (0.03 μm), latex spheres (1 μm) and bacteria (2.5 μm) have been shown

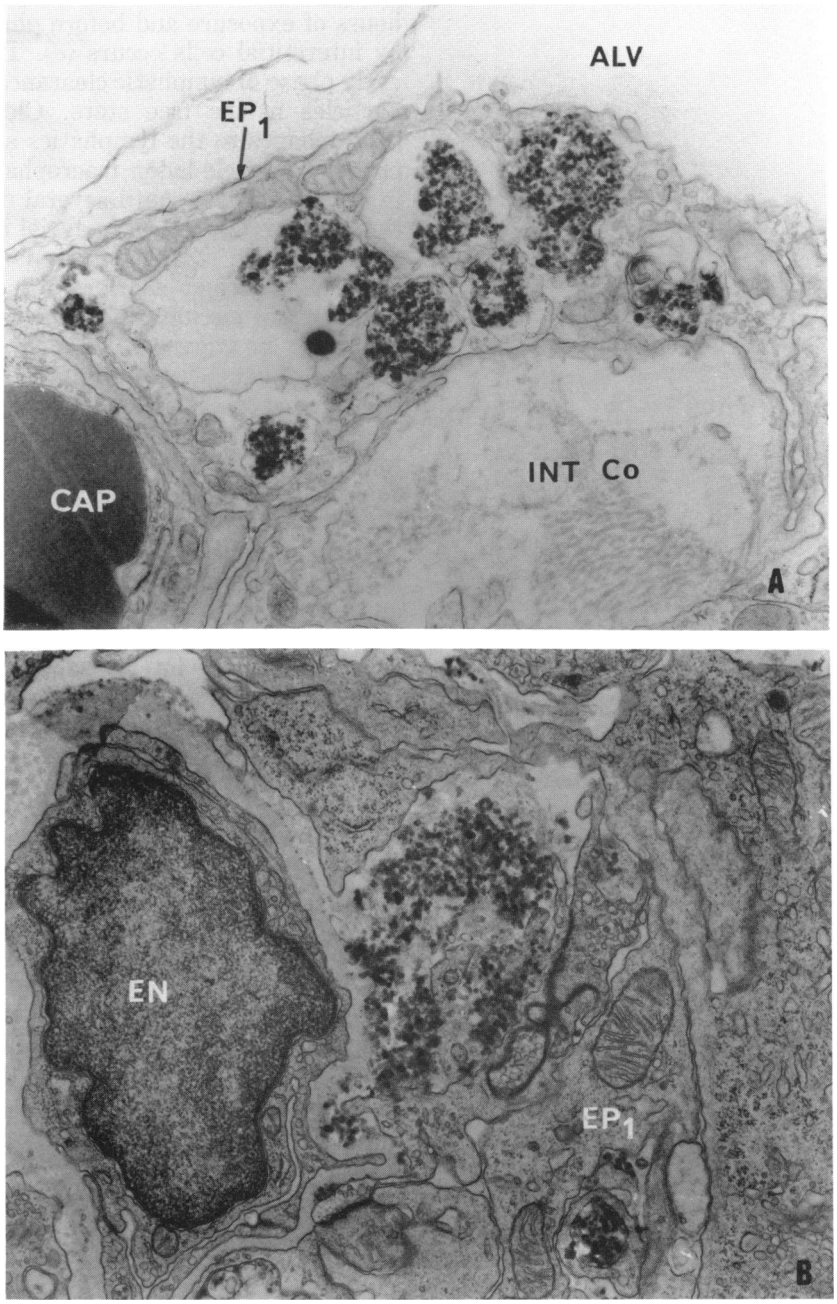

FIGURE 13. Micrographs of alveolar wall of a mouse (*A*) 6 hr after the instillation of carbon, particles of carbon are observed within cytoplasmic vacuoles of the Type I cell (EPI), but none is seen in spaces between the interstitial collagen (INT Co) nor in the capillary (CAP); (B) 12 hr after carbon, free particles lie in the interstitium between the Type I epithelium (EPI) and the endothelium (EN).

to cross the Type I epithelial cells within cytoplasmic vesicles (Fig. 13). Once across the barrier, the free particles may be phagocytosed by interstitial macrophages. With heavy and continued dust loading these cells tend to accumulate in the peribronchiolar spaces (Fig. 14). Such deposits may remain, apparently unchanged, for many years although the persistence of pigment laden cells in the sputum of ex-miners long after they have left the coal face indicates continuing, if slow, clearance. The process may be greatly accelerated by the massive infiltration of phagocytes which accompanies pulmonary infection; a bout of pneumonia may completely clear one or more lobes of a miner's black lung.

Inhaled particulates also reach the lymphatics. After the inhalation of small particles such as carbon, there appears to be rapid passage of some of these particles across the Type I cells to the interstitium and hence to the lymphatics (*8*). Carbon is seen in macrophages

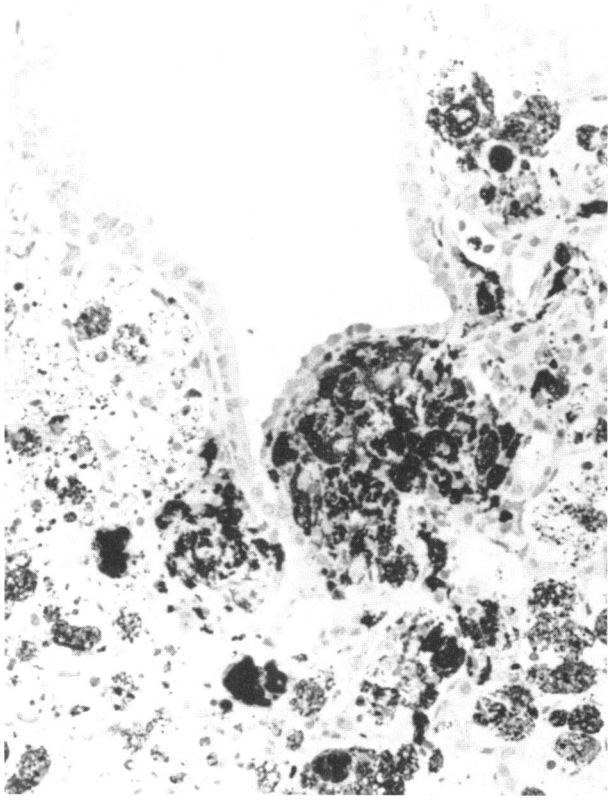

FIGURE 14. Methacrylate section 10 days after carbon showing a peribronchiolar nodule of carbon mostly within macrophages. Many carbon-laden macrophages are seen in neighboring alveoli; none is seen in bronchial epithelial cells. From Adamson and Bowden (*8*).

lining the sinusoids of hilar lymph nodes within several hours of exposure and before phagocytosis of particles by interstitial cells occurs (*8*). This suggests that the early phase of lymphatic clearance involves transport of particles in the free state. Clearance of interstitial macrophages to the lymphatics appears to be a slower process. Particle-laden macrophages are not observed in lymph channels until several days after administration and the amount of material removed by this route appears to be small compared to that eliminated by alveolar–bronchial clearance (*8*).

In certain circumstances, regular clearance mechanisms may be impaired. An agent such as silica destroys the macrophage, causing release of particles which then are engulfed by other macrophages. This process decreases clearance and facilitates transepithelial passage of the toxic material to the interstitium. Pollutant gases such as ozone and nitrogen dioxide, which selectively injure the respiratory bronchioles, cause necrosis at that site, thereby inhibiting the transfer of macrophages from alveolus to bronchus. Ciliostatic agents such as tobacco and alcohol may also inhibit clearance. Since alveolar movement is an important factor in macrophagic clearance, conditions such as interstitial edema and fibrosis, which reduce pulmonary compliance, are often associated with accumulations of macrophages apparently trapped in distal air sacs. These cells appear viable but usually are very large and the cytoplasm contains many vacuoles and debris, features characteristic of "old" macrophages (Fig. 15).

In summary, the most important route for clearance of pulmonary macrophages is the mucociliary pathway. Clearance by lymphatics appears to be of minor

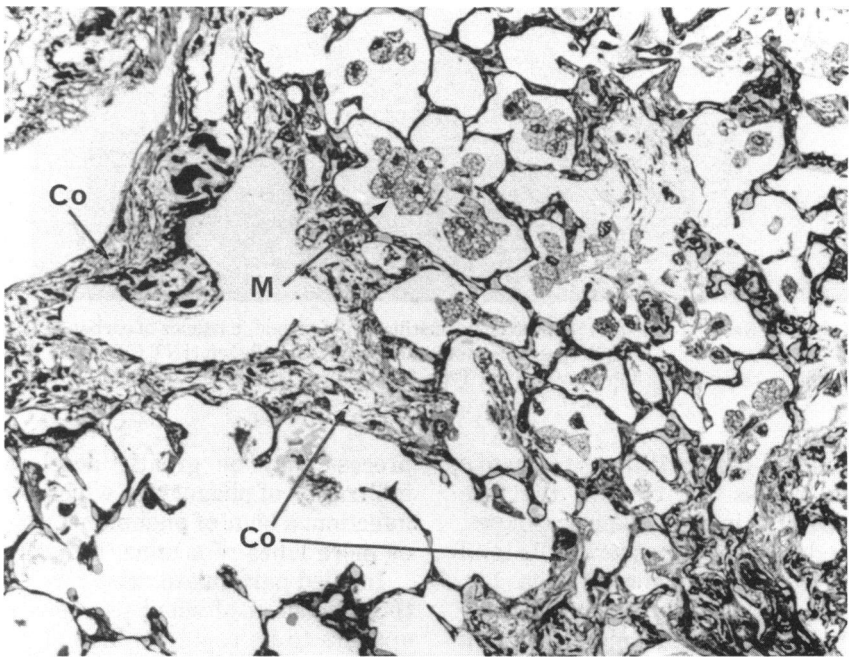

FIGURE 15. Large vacuolated macrophages (M) trapped in the alveoli of a mouse. The alveolar walls are thickened by bands of collagen (Co).

significance and there is no evidence that free alveolar macrophages recross the epithelium to reach the interstitium. Particulate matter that does reach the interstitium is transported across the epithelium in the free state and is phagocytosed by interstitial macrophages.

Abnormal Responses

The ability of the lungs to eliminate toxic or allergenic particles is dependent upon the integrated functioning of mucociliary clearance and the phagocytic activity of the alveolar macrophages. Most macrophagic reactions to inhaled particulates should be regarded as adaptive rather than pathological. Pathological changes may ensue if this adaptive capacity is overwhelmed or if the defensive functions of the macrophages are perturbed. Although the protective role of the macrophage must be considered dominant, it is possible that this cell participates directly or indirectly, in the genesis of two major groups of disabling lung diseases, chronic pulmonary fibrosis and emphysema. Such inappropriate responses involve interactions with fibroblastic cells and tissue injury initiated by macrophagic secretions.

Macrophage–Fibroblast Interactions

Acute or chronic injuries to the lung with their accompanying inflammatory responses may so impair the reparative process that fibrosis results. The immediate cause is rarely known but, sometimes, the macrophage may play at least an intermediary role. Phagocytosis of immune complexes as in the various types of allergic alveolitis, initiates the synthesis and release of lysosomal enzymes which may provide a continuing stimulus to fibroblastic secretion of collagen and the ultimate development of interstitial pulmonary fibrosis (*39*).

A more direct pathway of intercellular communication has been elucidated in the response of the lung to silica. The release of a fibrogenic principle by the dying macrophage is thought to stimulate the fibroblast directly (*40*). Since this reaction occurs within the interstitium, it is dependent upon the toxic particles crossing the epithelial barrier. The repeated sequence of phagocytosis, cell death and release of siliceous particles into the air sacs increases the likelihood of silica crystals being taken up by the Type I cells and transported to the interstitium; there the macrophagic granuloma of silicosis develops (Fig. 16 and 17). It is possible that such fibroblastic stimulation by injured macrophages represents a general mechanism (*41*). This is certainly true in extrapulmonary situations; for instance, it has been shown that release of macrophagic products in response to the subcutaneous injection of streptococcal components induces a chronic inflammatory response and fibrosis (*42*).

That intervention of the macrophage may not be essential to the genesis of pulmonary fibrosis is suggested by the response to asbestos. In asbestosis, the predominant location of fibrosis in the bronchiolar regions appears to be related to direct injury by fibers too long to be phagocytosed (*43*). Short fibers, < 20 μm in length, are seen frequently within phagosomes of macrophages (Fig. 18), but in contrast to silica, the cells are not destroyed. This difference is attributed to coating of the asbestos fibers by an iron-containing protein (*44*). These cells are cleared in the usual way and are not associated with the development of fibrosis.

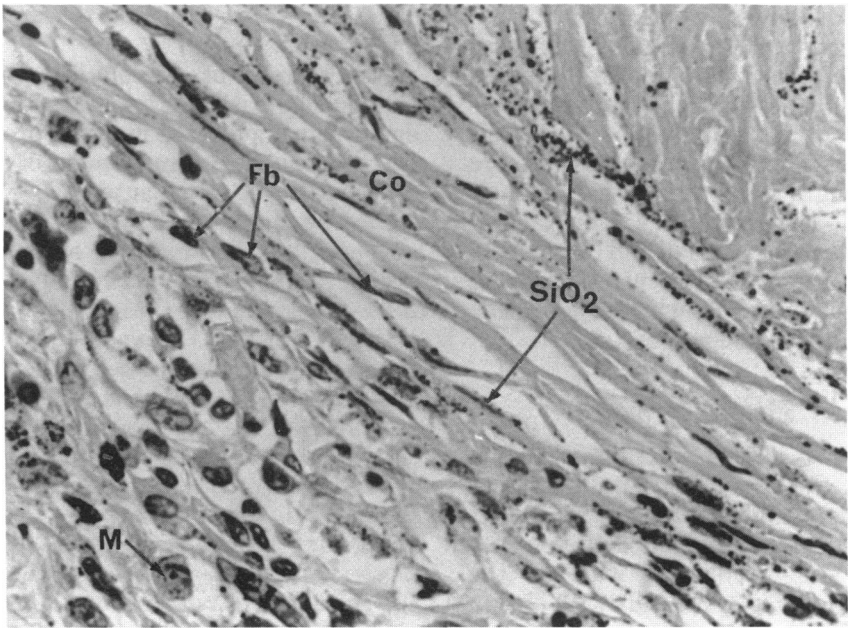

FIGURE 16. Silicotic nodule from the lung of a hard rock miner. At the margin of the fibrous nodule, macrophages (M) and fibroblasts (Fb) are closely associated.

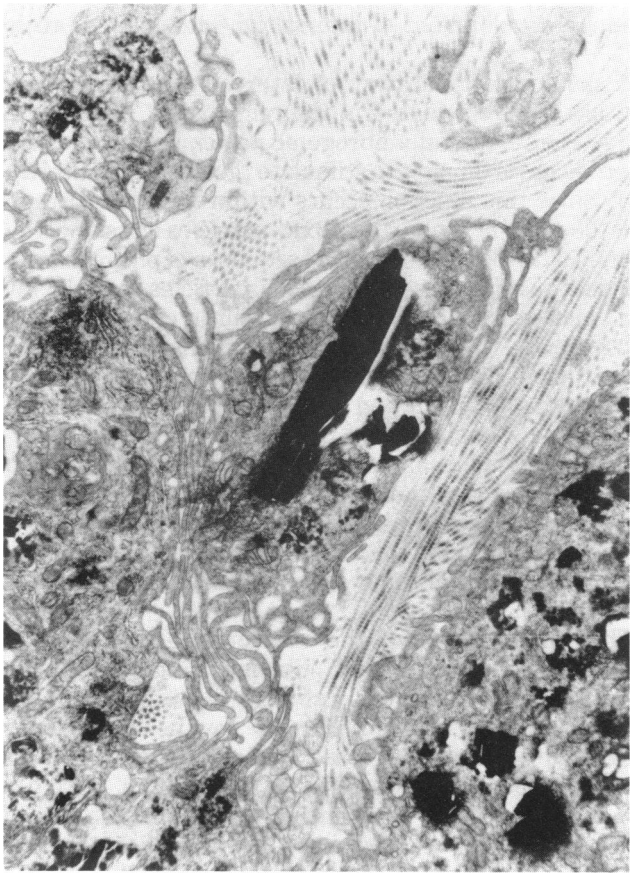

FIGURE 17. Electron micrograph showing the cellular constituents of a silicotic nodule. A spicule of silicotic material lies within the cytoplasm of a macrophage which is surrounded by collagen fibers.

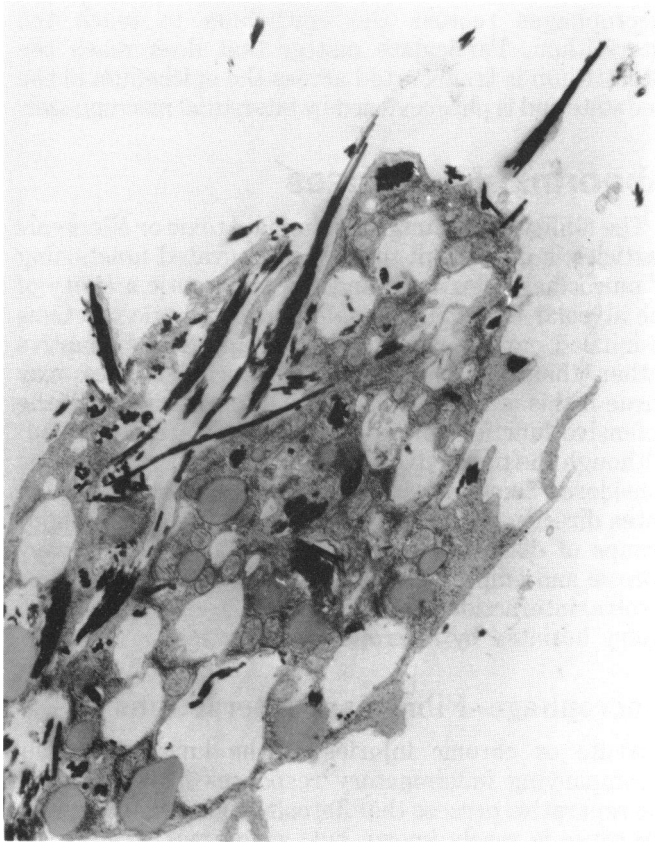

FIGURE 18. Electron micrograph demonstrating short < 5 μm fibers within a macrophage. Long fibers > 20 μm are excluded from the cell.

A similar sorting of fibers occurs with particles of glass fiber. Long thin fibers are most damaging, whereas shorter fibers are phagocytosed and effectively cleared by the macrophages (*45*). The work of Stanton and his colleagues (*46*) suggests that the induction of fibrosis and neoplasia by various fibrous particulates is more closely related to the length of the fibers than to their chemical composition. It is possible, however, that *in vivo*, the dichotomous response to small and large fibers may be less a function of fiber length than a result of effective filtering by macrophages which engulf and remove the majority of the smaller fibers.

Macrophage-Induced Tissue Injury

Phagocytosis and lysosomal activity are basic defensive functions of the macrophage. The possibility that such functions are associated on occasion with external secretory activity has been recognized only recently (*23,24*). It has been suggested that whereas the quiescent macrophage is occupied primarily with phagocytosis and endogenous digestion, an activated macrophage is capable of secreting enzymes that may be damaging to cellular and tissue components in its immediate vicinity.

As a secretor of proteolytic enzymes, the pulmonary macrophage has been implicated in the pathogenesis of emphysema, a disease in which progressive destruction and subsequent rearrangement of elastic fibers leads to an increasingly compliant lung. Similar lesions may be induced experimentally by the infusion of proteolytic enzymes into the lung and, since macrophages contain similar enzymes, it is tempting to suggest that their release may induce pulmonary autodigestion. Enzymatic secretion by the macrophage usually follows some form of extrinsic stimulus and, since cigarette smoking is a dominant factor in the etiology of emphysema, the response of the macrophage to tobacco smoke is of considerable interest.

The alveolar macrophages of cigarette smokers certainly are different from those of nonsmokers. Bronchopulmonary lavage yields four times as many macrophages from smokers than are obtained from nonsmokers; the smokers' cells are larger, their glucose utilization is increased about three times over control levels, and they exhibit distinctive morphologic features (*47*). Observed

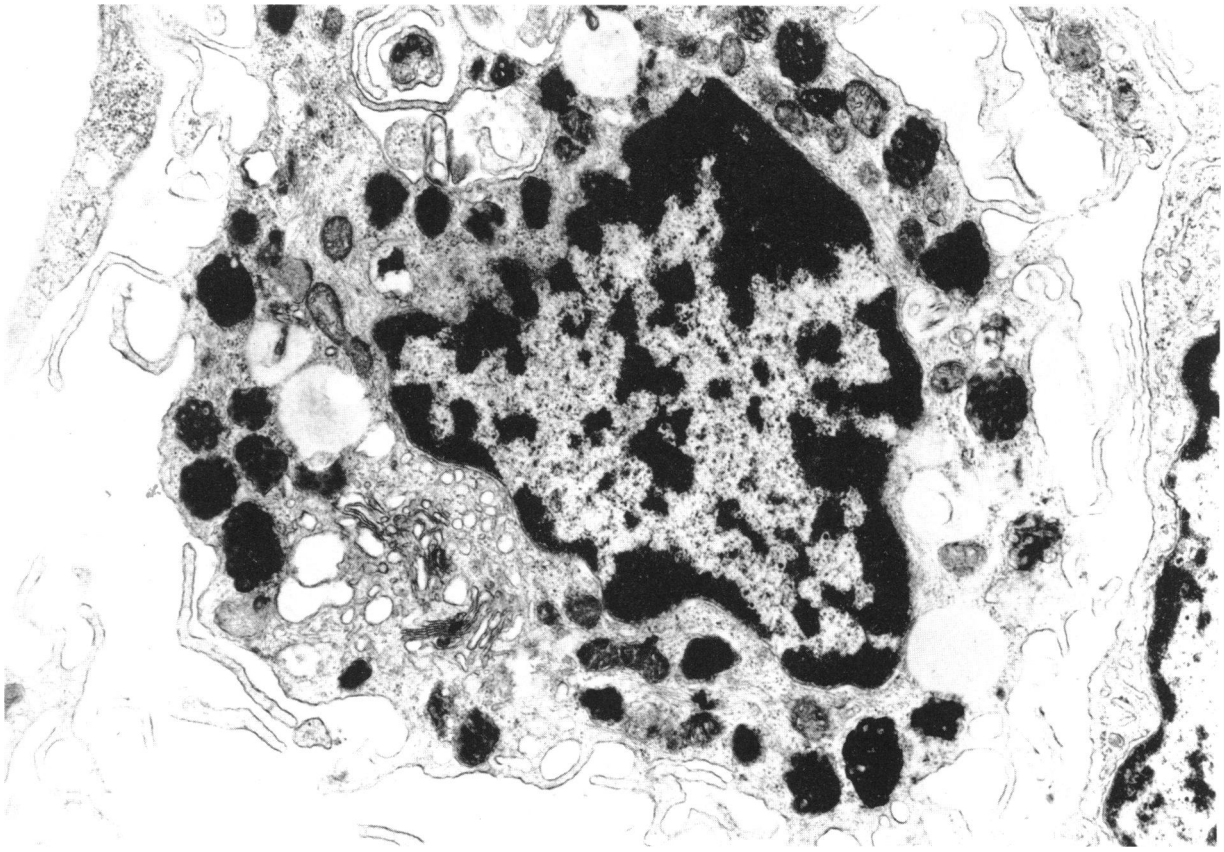

FIGURE 19. Alveolar macrophage seen in the alveolus of a "smoker's" lung. The cytoplasm exhibits a well-developed Golgi and many large, dense phagolysosomes.

with the electron microscope, the cells contain membrane-bound phagolysosomes containing acid phosphatase, there is an increase of endoplasmic reticulum and the Golgi is unusually prominent (*47,48*) (Fig. 19). The phagolysosomes, which are seen in both alveolar and interstitial macrophages, often contain deposits of kaolinite and aluminum silicate derived, probably, from inhaled tobacco smoke (*49*).

The macrophagic changes induced by tobacco smoke probably represent adaptive responses of the delivery system and of individual cells to an increased load. In this respect the boost in macrophage production is not unusual and it is reversible. At the functional level smokers' macrophages are competent phagocytes and they are capable of killing and digesting bacteria (*47*). It is tempting to conclude from this that the outpouring of competent macrophages in response to smoking represents an effective adaptive response. It is relevant to ask, however, whether the long-term cost of adaptation by the macrophagic system may not compromise the defenses of the host, contributing thereby to the development of chronic bronchitis.

Activated macrophages with their secretory potential may also be involved in the destruction of terminal airways and air sacs, leading to emphysema. Proteases with elastaselike activity have been demonstrated in macrophages from various sites and in smokers, alveolar macrophages exhibit greatly enhanced activity of this enzyme (*50*). Whether these proteolytic enzymes are capable of digesting collagen and elastic fibers of the lung is not known. It is difficult to comprehend how release of relatively low levels of digestive enzymes within the alveolar lumen could destroy, selectively, the spiral elastic fibers located in the centrilobular interstitial tissues. On the other hand, it is conceivable that free particulate matter reaching the interstitium could induce secretory activity in interstitial macrophages located close to these elastic fibers. Current enthusiasm for a macrophagic role in destructive diseases of the lung should not obscure the protective role of this cell. Neutrophil granulocytes, often observed in the interstitium and in the lung washings of cigarette smokers, carry a large complement of neutral proteases. Macrophages are capable of binding, incorporating and neutralizing such enzymes (*51,52*), and it is not unusual to observe fragments of neutrophilic leukocytes or even whole cells that have been phagocytosed by macrophages (Fig. 20). Once again, the protective role of the

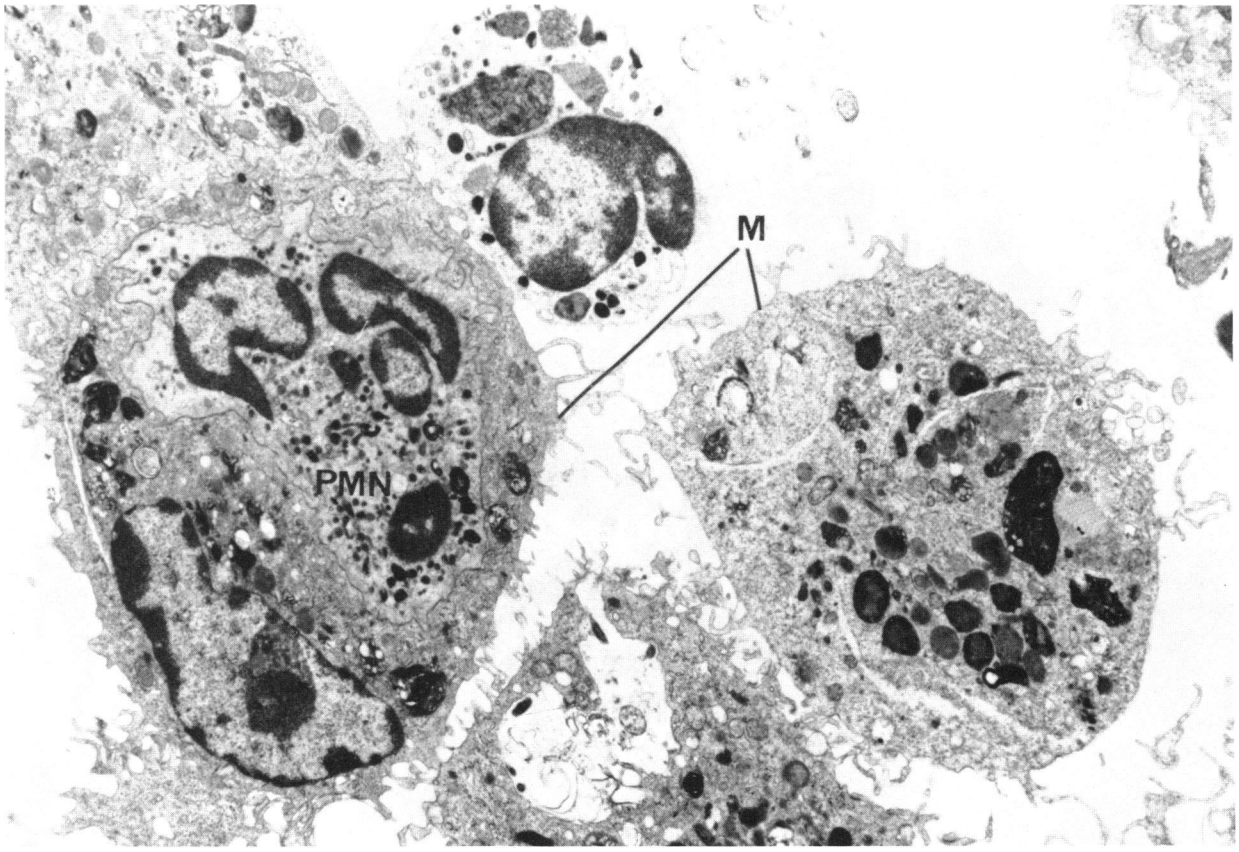

FIGURE 20. Two alveolar macrophages are shown (M); one has phagocytosed an intact polymorphonuclear leukocyte (PMN).

pulmonary macrophage appears to be dominant. Tissue injury, perpetuation of inflammatory responses, and other less desirable activities may occur under certain unusual circumstances, but the significance of these events fades when compared with the effective defensive pulmonary screen provided by the alveolar macrophage.

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